REMARKS/ARGUMENTS

By the present amendment, claims 4, 7, 9-10 and 12-14 have been amended rendering claims 1, 4-10, 12-16, 18-20, 24-30, 41, and 43-44 pending in the application. The amendments to the clams have been made without prejudice and without acquiescing to any of the Examiner's objections. Applicant reserves the right to pursue any of the deleted subject matter in a further continuation, continuation-in-part or divisional application. The amendment does not contain new matter and its entry is respectfully requested.

The Official Action dated April 29, 2004 have been carefully considered. It is believed that the amended claims submitted herewith and the following comments represent a complete response to the Examiner's rejections and place the present application in condition for allowance. Reconsideration is respectfully requested.

Claim Objections

Claims 4, 7, 9-10 and 13-14 have been amended as requested by the Examiner.

Claim 12 has been amended as requested by the Examiner.

35 USC §112, Second Paragraph

The Examiner has objected to Claims 1, 4-10, 12-16 and 18-19 under 35 USC §112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which the applicant regards as the invention. We respectively disagree for the following reasons.

As described in the specification (page 5), the term "pro-peptide" has been adequately defined in the application to mean the amino terminal portion of a zymogen or a functional portion thereof up to the maturation site. This term is definite and would be understood by a person of ordinary skill in the art. We respectively submit that the Examiner does not need to further interpret the term.

In view of the foregoing, we respectfully request that the objection to Claims 1, 4-10, 12-16 and 18-19 under 35 USC §112, second paragraph be withdrawn.

35 USC §112, First Paragraph

The Examiner has objected to Claims 10 and 16 under 35 USC §112, first paragraph because the specification, while being enabling for the method of claims 1 and 13, wherein the addition of the mature aspartic protease or chymosin in step c) takes place *in vivo* by transforming the host cell with an expression vector encoding the mature aspartic protease or chymosin and co-expressing the mature aspartic protease or chymosin in the host cell, does not reasonable provide enablement for the method of Claims 1 and 13, wherein the mature aspartic protease or chymosin is added to the fusion protein *in vivo* conditions by any means. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims. We respectively disagree for the following reasons.

The Applicant submits that multiple methods for adding the mature aspartic protease *in vivo* are disclosed in the application. The first method includes the addition of the mature aspartic protease or chymosin in step c) by transforming the host cell with an expression vector encoding the mature aspartic protease or chymosin and co-expressing the mature aspartic protease of chymosin in the host cell (page 12, line 27 to page 13, line 6). Another embodiment includes a therapeutic or nutritional peptide or protein that is administered to a mammal as an inactive fusion protein (i.e. as a pro-peptide fused to the therapeutic or nutritional peptide). The therapeutic or nutritional peptide or protein is administered *in vivo* to a mammal and activation or maturation through cleavage would occur upon its delivery at the unique physiological conditions prevalent at the target organ, tissue or bodily fluid, for example in the mammalian stomach, gut, kidneys, milk or blood. Furthermore, the mature form of chymosin or an inactive precursor form of chymosin may be added to assist in the cleavage of the nutritional or therapeutic peptide. (see page 13, lines 7 to 21). An example of *in vivo*

cleavage can be found in Example 3. Example 3 disclosed the cleavage of a Histidine-prochymosin-carp growth hormone fusion protein cleaved with the gut extract from red turnip beetle. In this example the extracts prepared from the gut of red turnip beetle cleaved the fusion protein and released the carp growth hormone polypeptide We respectively submit that the application includes a working example of *in vivo* cleavage.

In view of the foregoing, we respectfully request that the objection to Claims 10 and 16 under 35 USC §112, first paragraph be withdrawn.

The Examiner has objected to Claims 12 and 18 under 35 USC §112, first paragraph as failing to comply with the enablement requirements. The Examiner alleges that the claims contain subject matter which is not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it most nearly connected, to make and/or use the invention. We respectively disagree for the following reasons.

As described above, Example 3 disclosed the cleavage of a Histidine-prochymosin-carp growth hormone fusion protein cleaved with the gut extract from red turnip beetle. In this example, the extracts prepared from the gut of red turnip beetle cleaved the fusion protein and released the carp growth hormone polypeptide. We respectively submit that the application includes a working example of *in vivo* cleavage.

In view of the foregoing, we respectfully request that the objection to Claims 12 and 18 under 35 USC §112, first paragraph be withdrawn.

35 USC §102

The Examiner has rejected Claims 1, 4, 6-9, 13, 15 and 19 under 35 USC §102(b) as being anticipated by Walsh *et al.* (J. Biotech 45:235-241). We respectively disagree for the following reasons.

As the Examiner has stated, Walsh et al. teaches an expression vector encoding a fusion protein having a linker comprising a Phe-Met chymosin cleavage site (page 237). Walsh et al. teach a method for producing the encoded fusion protein by transforming a host cell with the fusion expression vector, culturing the resulting transformant to express the protein, followed by cleavage of the fusion protein at pH 4 and 5.8 by the addition of chymosin (page 236). First, we respectively submit that the specification indicates that the Phe-Leu amino acid junction is the pseudochymosin cleavage site and not the pro-chymosin cleavage site Phe-Val (see page 19 of the specification, top). We respectively submit that the Phe within the Phe-Met chymosin cleavage site, which is based on the k-casein cleavage site, does not constitute a chymosin-pro-peptide. Using BLAST analysis (http://www.ncbi.nlm.nih.gov/BLAST/) the amino acid sequence of the full-length chymosin pro-peptide (as shown in Figure 1 of the specification) was compared to the CL2 k-casein cleavage site from Walsh *et al.* No significant sequence similarity was observed between the two sequences. Since there is no significant sequence similarity, a person of ordinary skill in the art would not consider the k-casein cleavage site to be a functional chymosin pro-peptide. Consequently, the claims here within are not anticipated by Walsh et al.

In view of the foregoing, we respectfully request that the objection to the claims under 35 USC §102(b) be withdrawn.

35 USC §103

The Examiner has rejected claims 1, 4, 6-10, 13-16 and 19 under 35 USC §103(a) as being unpatentable over Ward *et al.* (US Patent 6,265,204 B1) in view of McCaman *et al.* (J. Biol. Chem. 261:15345-15348). We respectfully disagree with the Examiner for the reasons that follow.

We respectively submit that the inventive step of the present claims involves the recognition that if a chymosin pro-peptide/heterologous protein fusion is created and a mature form of an autocatalytically maturing aspartic protease that is capable of cleaving the chymosin pro-peptide is added to the fusion protein, that cleavage of the

protein fusion would result. As the Examiner has noted, Ward et al. does not add chymosin to effect cleavage of the fusion protein.We respectively disagree with the Examiner's assertion that at the time of the invention, one of ordinary skill in the art would have recognized that chymosin is an appropriate endoproteinase for cleaving a fusion protein comprising a chymosin prosequence. At the time of the invention, one of ordinary skill in the art would recognize that pro-chymosin is autocatalytically processed at pH 4.5 but would not have recognized that a chymosin pro-peptide could be cleaved by the addition of the mature form of an autocatalytically maturing aspartic protease from a heterologous peptide and expect success. In view of the fact, as the examiner points out, that mature chymosin was not added (Ward *et al.*), a person of ordinary skill in the art would not necessarily expect success. Three reasons why a person of ordinary skill in the art may not expect success include: 1) not knowing if an intact polypeptide would result with the addition of mature chymosin (i.e. would there be non-specific cleavage of the heterologous polypeptide); 2), it was unknown whether or not the addition of mature chymosin would result in undesired overhangs (i.e. not precise cleavage) and; 3) it was not known whether or not the addition of mature chymosin would be efficient (i.e. would a significant amount of the heterologous protein be cleaved).

We remind the Examiner that he is to consider secondary considerations when assessing obviousness. Secondary considerations relevant to the present invention include long-felt need in the art. The expression of valuable recombinant proteins (such as therapeutic proteins) is highly desirable. However, as stated previously, there are difficulties recognized in the art with respect to the efficient production and recovery of recombinant proteins. In fact Ward *et al.* teaches (column 14, lines 33-48) that in some embodiments, after cleavage via chemicals or endoproteinases, the desired polypeptides contain unwanted amino acids from the amino or carboxy termini and that a variety of aminopeptidases and carboxypeptidases of differing specificities may be used to remove the amino acids from the cleavable linker. The present invention solves the difficulties of the prior art by providing an efficient method of cleaving recombinant proteins. Further, the present invention permits the use of fusion proteins for larger

scale manufacturing, both by reducing the cost of the cleavage agent and by addressing safety issues arising from the use of certain cleavage agents. Therefore, there is a clear long-felt need in the art for the present invention which must be given due weight when considering inventive step.

The deficiencies in Ward *et al.* are clearly not remedied by McCaman (1986) as McCaman *et al.* (1986) teaches the effect of amino acid changes of the autocatalytic processing of pro-chymosin.

The Examiner has rejected claim 5 under 35 USC §103(a) as being unpatentable over Ward *et al.* in view of McCaman *et al.* as applied to claims 1, 4, 6-9, 13, 15 and 19 above and further in view of Fine *et al.* (*Gen Comp Endocrinol* 89:51-61). We respectfully disagree with the Examiner for the reasons that follow.

Claim 5 relates to a specific embodiment of the invention wherein the heterologous protein is carp growth hormone or hirudin. As a result, these claims carry with them all of the novel and inventive features of Claim 1 from which this claim depends. Our comments on the Ward *et al.* and McCaman *et al.* references appear above and equally apply to this claim. The deficiencies in the Ward *et al.* and McCaman *et al.* references are in no way remedied by Fine *et al.* which is a reference that describes expression of Carp Growth Hormone (cGH) in *E.coli*, purification of cGH from *E.coli*, *in vitro* (using lymphoma and preadipocyte cells) and *in vivo* (evaluating growth rate in fish injected with the purified protein) characterization of cGH. Fine *et al.* in no way teaches or suggests an improved method to prepare cGH by linking the cGH to a pro-peptide from chymosin.

The Examiner has rejected claim 5 under 35 USC §103(a) as being unpatentable over Walsh *et al.* in view of Fine *et al.* (*Gen Comp Endocrinol* 89:51-61). We respectfully disagree with the Examiner for the reasons that follow.

Claim 5 relates to a specific embodiment of the invention wherein the heterologous protein is carp growth hormone or hirudin. As a result, these claims carry with them all of the novel and inventive features of Claim 1 from which this claim depends. Our comments on the Walsh *et al.* reference appear above and equally apply to this claim. The deficiencies in the Walsh *et al.* reference is in no way remedied by Fine which is a reference that describes expression of Carp Growth Hormone (cGH) in *E.coli*, purification of cGH from *E.coli*, *in vitro* (using lymphoma and preadipocyte cells) and *in vivo* (evaluating growth rate in fish injected with the purified protein) characterization of cGH. Fine *et al.* in no way teaches or suggests an improved method to prepare cGH by linking the cGH to a pro-peptide from chymosin.

The Examiner has rejected to Claim 14 under 35 USC §103(a) as being unpatentable over Walsh *et al.* in view of Dunn *et al.* ("Aspartic Proteinases", Advances in Experimental Medicine and Biology, Volume 362, Plenum Press, NY, 1995, pages 1-9) OR Ward *et al.* in view of McCaman *et al.* as applied to Claims 1, 4, 6-9, 13, 15 and 19 above and further in view of Dunn *et al.* We respectfully disagree with the Examiner for the reasons that follow.

Claim 14 relates to a specific embodiment of the invention wherein the aspartic protease added in step c) is heterologous to chymosin. As a result, these claims carry with them all of the novel and inventive features of Claim 1 from which this claim depends. Our comments on the Walsh *et al.* reference appear above and equally apply to this claim. The deficiencies in the Walsh *et al.* reference is in no way remedied by Dunn *et al.* which is a reference that describes the cleavage of a peptide by a number of aspartic proteases including porcine pepsin, human cathepsin D and the aspartic protease from *Rhizopus chinesis.* It should be noted that the peptide is a synthetic peptide that has no significant homology to the propeptide of the aspartic proteases (http://www.ncbi.nlm.nih.gov/BLAST/). A person of ordinary skill in the art using the teachings of Dunn *et al.* would realize that a synthetic peptide containing a phenylalanine amino acid residue could be cleaved by a number of aspartic proteases

but this reference in no way teaches that aspartic proteases would be able to cleavage a chymosin pro-peptide.

The Examiner has rejected to Claim 14 under 35 USC §103(a) as being unpatentable over Ward *et al.* in view of McCaman *et al.* as applied to Claims 1, 4, 6-9, 13, 15 and 19 above and further in view of Dunn *et al.* We respectfully disagree with the Examiner for the reasons that follow.

Claim 14 relates to a specific embodiment of the invention wherein the aspartic protease added in step c) is heterologous to chymosin. As a result, these claims carry with them all of the novel and inventive features of Claim 1 from which this claim depends. Our comments on the Ward *et al.* in view of McCaman *et al.* references appear above. The deficiencies in the Ward *et al.* in view of McCaman *et al.* references are in no way remedied by Dunn *et al.* which is a reference that describes the cleavage of a peptide by a number of aspartic proteases including porcine pepsin, human cathepsin D and the aspartic protease from *Rhizopus chinesis*. As described above, a person of ordinary skill in the art using the teachings of Dunn *et al.* would realize that a synthetic peptide containing a phenylalanine amino acid residue could be cleaved by a number of aspartic proteases but this reference in no way teaches that aspartic proteases would be able to cleavage a chymosin pro-peptide.

In view of the foregoing, we respectfully request that all of the objections under 35 USC §103 be withdrawn.

The Commissioner is hereby authorized to charge any deficiency in fees (including any claim fees) or credit any overpayment to our Deposit Account No. 02-2095.

In view of the foregoing, we submit that the application is in order for allowance and an early indication to that effect would be greatly appreciated. Should the Examiner like to discuss the matter, he is kindly requested to contact Micheline Gravelle at 416-957-1682 at his convenience. We remind the Examiner that this case has been pending at

the United States Patent Office greater than 4.5 years so early resolution of the outstanding issues is respectfully requested.

Respectfully submitted,

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Attachments